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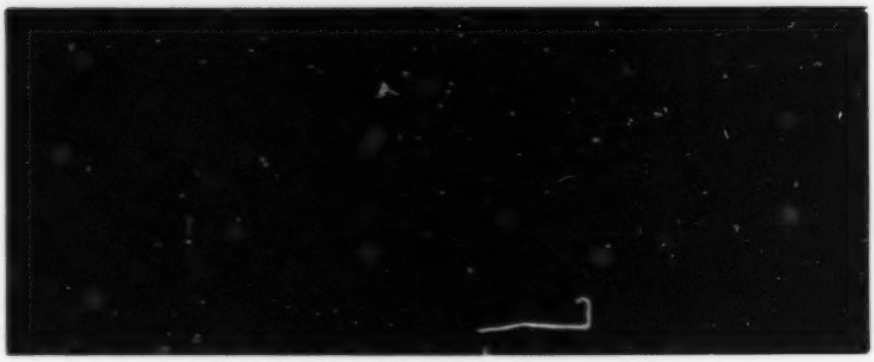

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Study of a Bleeding Disorder of Simmental Cattle

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Study of a Bleeding Disorder of Simmental Cattle

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Background & Objectives

A bleeding disorder of Simmental cattle has been recognized in Western Canada since 1972 and since 1980 several of these animals have been presented to the Western College of Veterinary Medicine.[1] Owners of affected animals report prolonged spontaneous nasal hemorrhage and less frequently urinary tract hemorrhage. These animals become debilitated because of iron depletion secondary to compensatory red-cell production. Prolonged hemorrhage has occurred following minor surgery and death has resulted from major surgery.

Laboratory evaluation of these animals as reported in previous submissions has revealed that this bleeding disorder is the result of impaired platelet function. The objective of this study was to further elusidate the molecular basis of impaired platelet function responsible for persistent hemorrhage in affected Simmental cattle.

Experimental Methods

Platelet Aggregation

Thirty-eight normal cattle including Simmental cattle and five affected Simmental cattle were studied. A whole blood aggregometer system (a) was used and platelet aggregation was assessed in response to 5 ul of collagen and 3 ul of ADP. The results were expressed as percent aggregation at 10 minutes following addition of the agonists.

Platelet Electrophoresis

Platelet rich plasma was harvested from normal cattle and an affected cow.

Blood was taken by jugular venipuncture directly into acid-citrate-dextrose as previously described.[2] Platelet suspensions were prepared, subjected to single dimensional sodium dodecyl sulfate (SDS) - polyacrylamide gel electrophoresis and stained as described by Nurden et al.[2]

Results

Platelet Aggregation

There was a marked contrast in aggregation patterns between normal and bleeding Simmental cattle. There was consistently greater than 50% aggregation of platelets in response to ADP and collagen in normal cattle and less than 10% to both agonists in affected Simmental cattle.

(a) Whole Blood Aggregometer Model 500, Chrono-Log Corp. Havertown Pa.

P atelet Membrane Electrophoresis

Analysis of platelet proteins was performed on 7-12% gradient acrylamide slab gels allowing detection of the major polypeptides within a molecular weight range of 400,000 to 20,000. The polypeptide profiles of the platelets of one affected cow and one normal cow are illustrated in Fig. 1. No gross protein abnormalities were observed.

Discussion

Congenital bleeding disorders in man associated with impaired platelet aggregation are known as Glantzman's thrombosthenia.[3] Recently this has been recognized as a heterogeneous group of disorders involving one combination of platelet membrane glycoproteins known as the IIb-IIIa complex.[4] In normal stimulated platelets this complex binds to fibrinogen and fibronectin, large molecular weight proteins. Following binding these proteins serve as bridging molecules between adjacent platelets bringing about aggregation.[4] Large clumps of aggregated platelets are essential to blood clotting and repair of damaged vessels. They result in a physical plugging of the damaged vessel wall and also release their contents into the surrounding medium. They play an important role in blood coagulation which results in the deposition of fibrin in the platelet plug stabilization of the plug and eventually repair.

Our preliminary work would suggest that the congenital bleeding disorder in Simmental cattle closely resembles Glantzman's Thrombosthenia. The failure of platelets from affected cattle to aggregate in response to agonists such as collagen and ADP would suggest that binding of fibrinogen to platelets may be impaired. This brings into question the integrity of the receptor on the platelet membrane to which fibrinogen normally binds. The electrophoretic studies are an attempt to isolate, stain and visualize groups of platelet proteins according to their molecular weights and surface changes. The SDP-Page single dimensional electrophoresis method of this study revealed no gross protein abnormalities. Further studies using two dimensional SDS-Page electrophoresis are necessary in combination with the use of monoclonal antibodies[5] to those platelet membrane proteins responsible for fibrinogen binding.

Reference:

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